



Year: 2012

OSR1-sensitive renal tubular phosphate reabsorption

Pathare, Ganesh ; Föller, Michael ; Daryadel, Arezoo ; Mutig, Kerim ; Bogatikov, Evgeny ; Fajol, Abul ; Almilaji, Ahmad ; Michael, Diana ; Stange, Gerti ; Voelkl, Jakob ; Wagner, Carsten A ; Bachmann, Sebastian ; Lang, Florian

Abstract: Background: The oxidative stress-responsive kinase 1 (OSR1) participates in the WNK-(with no K) kinase dependent regulation of renal salt excretion and blood pressure. Little is known, however, about the role of OSR1 in the regulation of further renal transport systems. The present study analyzed the effect of OSR1 on NaPiIIa, the major renal tubular phosphate transporter. Methods: Immunohistochemistry and confocal microscopy were employed to determine renal localization of OSR1 and NaPiIIa. To elucidate the effect of OSR on NaPiIIa activity, cRNA encoding NaPiIIa was injected into *Xenopus* oocytes with or without additional injection of cRNA encoding OSR1, and phosphate transport was estimated from phosphate-induced currents determined with dual electrode voltage clamp. To elucidate the in vivo significance of OSR1 serum phosphate and hormone concentrations as well as urinary phosphate output of mice carrying one allele of WNK-resistant OSR1 (*osr1*(tg/+)) were compared to the respective values of wild type mice (*osr1*(+/+)). Results: NaPiIIa and OSR1 were both expressed in proximal renal tubule cells. Coexpression of OSR1 significantly up-regulated phosphate-induced currents in NaPiIIa-expressing *Xenopus* oocytes. Despite decreased serum phosphate concentration urinary phosphate excretion was significantly increased and NaPiIIa protein abundance in the brush border membrane significantly reduced in *osr1*(tg/+) mice as compared to *osr1*(+/+) mice. Serum PTH and calcitriol levels were similar in *osr1*(tg/+) mice and in *osr1*(+/+) mice, serum FGF23 concentration was, however, significantly higher in *osr1*(tg/+) mice than in *osr1*(+/+) mice. Conclusions: OSR1 is expressed in proximal renal tubules and participates in the regulation of FGF23 release and renal tubular phosphate transport.

DOI: <https://doi.org/10.1159/000343405>

Posted at the Zurich Open Repository and Archive, University of Zurich

ZORA URL: <https://doi.org/10.5167/uzh-68142>

Journal Article

Accepted Version

Originally published at:

Pathare, Ganesh; Föller, Michael; Daryadel, Arezoo; Mutig, Kerim; Bogatikov, Evgeny; Fajol, Abul; Almilaji, Ahmad; Michael, Diana; Stange, Gerti; Voelkl, Jakob; Wagner, Carsten A; Bachmann, Sebastian; Lang, Florian (2012). OSR1-sensitive renal tubular phosphate reabsorption. *Kidney Blood Pressure Research*, 36(1):149-161.

DOI: <https://doi.org/10.1159/000343405>

OSR1-sensitive renal tubular phosphate reabsorption

Ganesh Pathare¹, Michael Föller¹, Arezoo Daryadel², Kerim Mutig³, Evgeny Bogatkov¹, Abul Fajol¹, Diana Michael¹, Gerti Stange², Jakob Völkl¹, Carsten A. Wagner², Sebastian Bachmann^{3#}, Florian Lang^{1#}

¹Department of Physiology, University of Tübingen, Gmelinstr. 5, D-72076 Tübingen, Germany,

²Institute of Physiology and Zurich Center for Integrative Human Physiology, University of Zurich, Winterthurerstraße 190, 8057 Zürich, Switzerland

³Institut für Vegetative Anatomie, Charitéplatz 1, D-10117 Berlin, Germany

#contributed equally and thus share last authorship

Running title: OSR1-sensitive Pi transport

word count abstract: 249

word count text: 1623

Address for correspondence:

Florian Lang

Department of Physiology, University of Tübingen

Gmelinstr. 5, D-72076 Tübingen, Germany

phone +49-7071-2972194, fax: +49-7071-295618

e-mail: florian.lang@uni-tuebingen.de

Abstract

The oxidative stress-responsive kinase 1 (OSR1) participates in the WNK-(with no K) dependent regulation of renal salt excretion and blood pressure. Little is known, however, about the role of OSR1 in the regulation of further renal transport systems. The present study analyzed the effect of OSR1 on NaPiIIa, the major renal tubular phosphate transporter. Immunohistochemistry and confocal microscopy were employed to determine renal localization of OSR1 and NaPiIIa. To elucidate the effect of OSR on NaPiIIa activity, cRNA encoding NaPiIIa was injected into *Xenopus* oocytes with or without additional injection of cRNA encoding OSR1, and phosphate transport was estimated from phosphate-induced current determined with dual electrode voltage clamp. To elucidate the *in vivo* significance of OSR1 serum phosphate and hormone concentrations as well as urinary phosphate output of mice carrying one allele of WNK-resistant OSR1 (*osr1^{tg/+}*) were compared to the respective values of wild type mice (*osr1^{+/+}*). As a result, NaPiIIa and OSR1 were both expressed in proximal renal tubule cells. Coexpression of OSR1 significantly up-regulated phosphate-induced currents in NaPiIIa-expressing *Xenopus* oocytes. Despite a decreased serum phosphate concentration urinary phosphate excretion was significantly increased and NaPiIIa protein abundance in the brush border membrane significantly reduced in *osr1^{tg/+}* mice as compared to *osr1^{+/+}* mice. Serum PTH and calcitriol levels were similar in *osr1^{tg/+}* mice and in *osr1^{+/+}* mice, serum FGF23 concentration was, however, significantly higher in *osr1^{tg/+}* mice than in *osr1^{+/+}* mice. In conclusion, OSR1 is expressed in proximal renal tubules and participates in the regulation of renal tubular phosphate transport.

Key words: Na⁺, phosphate cotransporter, FGF23, bone density

Introduction

The oxidative stress-responsive kinase 1 (OSR1) participates in the signaling regulating transport during oxidative and osmotic stress¹⁻⁶. OSR1 is activated by the osmosensitive WNK (with no K) kinase isoforms WNK1 and WNK4^{3;7;8} and up-regulates the thiazide-sensitive Na⁺-Cl⁻ cotransporter (NCC) and the furosemide-sensitive Na⁺,K⁺,2Cl⁻ cotransporters (NKCC1 and NKCC2), thus contributing to the regulation of cell volume, transepithelial transport, renal salt excretion, and GABA neurotransmission^{1-3;9-14}. Presumably due to their impact on renal tubular salt transport WNK1 and WNK4^{2;15-18} and OSR1^{7;19-22} participate in the regulation of blood pressure.

Nothing is known about a role of OSR1 in other nephron segments and in other renal tubular transport systems. The present study thus explored whether OSR1 influences the activity of the Na⁺-coupled phosphate transporter NaPiIIa (SLC34A1), which is the most important carrier accomplishing renal tubular phosphate transport across the apical brush border membrane of proximal renal tubules²³⁻²⁵. Renal tubular phosphate reabsorption is tightly regulated by dietary phosphate intake, acid-base status, parathyroid hormone, 1,25-(OH)₂ vitamin D₃, FGF23, insulin and insulin-like growth factor IGF1²⁶⁻³². Signaling known to regulate NaPiIIa includes the protein kinases A and C, ERK1/2, Klotho and the PI3K/PKB/GSK3 kinase cascade³³⁻⁴⁰.

The present study addressed the putative role of OSR1 in the regulation of NaPiIIa. Coexpression of OSR1 enhanced phosphate-induced currents reflecting electrogenic phosphate transport in NaPiIIa-expressing *Xenopus* oocytes. Immunohistochemistry and confocal microscopy revealed coexpression of OSR1 and NaPiIIa in proximal renal tubules. Urinary phosphate excretion was higher and serum phosphate concentration lower in heterozygous OSR1 knockin mice resistant to WNK-mediated activation (*osr1^{tg/+}*) than in respective wild type mice (*osr1^{+/+}*). Thus, the observations point to a role of OSR1 in the regulation of renal tubular phosphate transport.

Materials and Methods

In vitro expression

For generation of cRNA, constructs were used encoding wild type human NaPiIIa⁴⁹ and human OSR1. The cRNA was generated as described previously⁵⁰. The constructs encoding wild type and catalytically-inactive D164A OSR1 were kindly provided by Dario Alessi (University of Dundee, UK). For electrophysiology, *Xenopus* oocytes were prepared as previously described^{51;52}. Fifteen ng of NaPiIIa cRNA were injected on the first day and 10 ng OSR1 cRNA on the second day into *Xenopus* oocytes. All experiments were performed at room temperature 3 days after the second injection. Two electrode voltage-clamp recordings were performed at a holding potential of -50 mV. The data were filtered at 10 Hz and recorded with a Digidata A/D-D/A converter and Chart V.4.2 software for data acquisition and analysis (Axon Instruments). The control solution (superfusate / ND96) contained 96 mM NaCl, 2 mM KCl, 1.8 mM CaCl₂, 1 mM MgCl₂ and 5 mM HEPES, pH 7.4. One mM phosphate was added to induce NaPiIIa-dependent currents. The flow rate of the superfusion was 20 ml/min, and a complete exchange of the bath solution was reached within about 10 s.

Animals

All animal experiments were conducted according to the German law for the welfare of animals and were approved by local authorities. Blood was drawn, urine collected or tissue isolated from sex- and age-matched 3-8-month-old heterozygous OSR1 knockin mice (*osr1^{tg/+}*) and respective wild type mice (*osr1^{+/+}*), kindly provided by Dario Alessi. As described earlier⁵³, in the knockin mice the T-loop Thr residue in OSR1 (Thr185) was mutated to Ala to prevent activation by WNK isoforms. Homozygous OSR1 knockin mice (*osr1^{tg/tg}*) are not viable⁵³. Mice had free access to control diet (sniff, Soest, Germany) containing 7000 mg/kg phosphorus or to phosphate-depleted diet (Altromin, Lage, Germany) containing 131 mg/kg phosphate and to tap drinking water ad libitum. To obtain serum, mice were anaesthetized with diethylether (Roth, Karlsruhe, Germany) and blood was withdrawn into capillaries by puncturing the retrobulbar plexus.

To determine creatinine clearance, urinary flow rate and urinary excretion of phosphate, the mice were placed individually in metabolic cages (Techniplast, Hohenpeissenberg, Germany) as described previously⁵⁴. They were allowed a 2 days habituation period during which food and water intake, urinary flow rate and phosphate excretion were recorded every day to ascertain that the mice were adapted to the new environment. Subsequently, 24 h collection of urine was performed for three consecutive days in order to obtain the urinary parameters. This procedure was repeated under low-phosphate diet. To assure quantitative urine collection, metabolic cages were siliconized, and urine was collected under water-saturated oil.

The phosphate concentration was determined colorimetrically utilizing a commercial diagnostic kit (Roche Diagnostics, Mannheim, Germany). The urinary concentration of Ca²⁺ was measured by flame photometry (Eppendorf, Hamburg, Germany). The creatinine concentration in urine was determined using the Jaffe reaction (Labor und Technik, Berlin, Germany). The serum intact parathormone concentration was measured using an ELISA kit (Immunotopics, San Clemente, USA) as was the concentration of 1,25(OH)₂D₃ (IDS Diagnostics, Frankfurt/Main, Germany) and of FGF23 (Immunotopics).

Brush border membrane vesicles (BBMV) preparation and immunoblotting

BBMVs were prepared from mouse kidney cortex and outer medulla using the Mg²⁺ precipitation technique as described previously^{55;56}. The total protein concentration was measured using the Bio-Rad Protein Assay kit (Bio-Rad, Hercules, CA, USA). BBMVs were stored at -80°C until further use. Five micrograms of renal brush border membrane proteins were solubilized in loading buffer containing dithiothreitol (DTT) and separated on 8% polyacrylamide gels. For immunoblotting, the proteins were transferred electrophoretically to polyvinylidene fluoride (PVDF) membranes (Immobilon-P, Millipore, Bedford, MA, USA). After blocking with 5% milk powder in Tris-buffered saline/0.1% Tween-20 for 60 min, the

blots were incubated with the primary antibodies: rabbit polyclonal anti-NaPiIIa (1:6000)⁵⁷ and mouse monoclonal anti- β -actin antibody (42 kD; Sigma, St. Louis, MO; 1:5000) either for 2 h at room temperature or overnight at 4°C. Membranes were then incubated for 1 h at room temperature with secondary goat anti-rabbit or donkey anti-mouse antibodies 1:5000 linked to alkaline phosphatase (Promega, Mannheim, Germany) or to horseradish peroxidase (HRP) (Amersham, Freiburg, Germany). The protein signal was detected with the appropriate substrates (Millipore, Schwalbach, Germany) using the DIANA III-chemiluminescence detection system (Raytest, Straubenhardt, Germany). All images were analysed using the software Advanced Image Data Analyser AIDA, Raytest to calculate the protein of interest/ β -actin ratio.

Western blotting of whole kidney

To determine protein abundance in renal tissue, mice were anesthetized with diethylether. The kidneys were removed and immediately shock-frozen in liquid nitrogen. Renal tissue was homogenised with an electric homogenizer at 4°C in lysis buffer (54.6 mM HEPES; 2.69 mM $\text{Na}_4\text{P}_2\text{O}_7$; 360 mM NaCl; 10% [vol/vol] Glycerol; 1% [vol/vol] NP40) containing phosphatase and protease inhibitors (Complete mini, Roche, Mannheim, Germany). Homogenates were clarified by centrifugation at 20000 g for 20 min. Total protein (100 μg) was separated by SDS-PAGE (10% Tris-Glycine), transferred to nitrocellulose membranes (Schleicher and Schuell, Dassel, Germany), blocked for 3 h in blocking buffer (5% fat-free milk in Tris-buffered saline (TBS) containing 0.1% Tween), and incubated overnight with an anti Klotho antibody (kindly provided by Akiko Saito from the Kyowa Hakko Kirin Co., Ltd., Japan) (1:1.000 in 5% fat free milk in TBS). After incubation with a horseradish peroxidase-conjugated anti-rat secondary antibody (Cell Signaling, Germany), the bands were visualized with enhanced chemiluminescence according to the manufacturer's instructions. Homogenates were also probed with a primary GAPDH (Cell Signaling) antibody as loading control. Densitometric analysis of Klotho and GAPDH was performed using Quantity One software (Bio-Rad Laboratories).

Immunohistochemistry

For immunofluorescence microscopic detection of NaPiIIa and OSR in proximal tubule immunolabeling was performed on cryostat or paraffin sections of 5 μm thickness, blocked with 5% milk powder in PBS, incubated for 2 h with primary antibodies (sheep anti-OSR antibody [1:500 dilution; University of Dundee] and rabbit anti-NaPiIIa [1:400 dilution; kind gift of J. Biber, Zurich]), followed by secondary antibodies (Cy3-coupled donkey-anti sheep IgG and Cy2-coupled swine-anti rabbit IgG [Dianova]). Specificity of the double-staining procedures was controlled by parallel incubation of consecutive sections, each incubated only with one single probe. Fluorescence signals were detected by confocal laser scanning microscopy (LSM 510 META; Carl Zeiss, Jena, Germany) with a Plan Neofluar 100 \times /1.3 oil objective.^{58;59}

Statistics

Data are provided as means \pm SEM, *n* represents the number of independent experiments. All data were tested for significance using unpaired Student's t-test or ANOVA, as appropriate. Only results with *p* < 0.05 were considered statistically significant.

Results

Immunohistochemistry was employed to explore whether OSR1 is expressed in proximal renal tubules. As illustrated in Fig. 1, OSR1 protein could indeed be detected in the proximal nephron. Concomitant labelling of the proximal tubular phosphate transporter NaPiIIa revealed co-localization of the kinase with the carrier in a subset of proximal tubules (Fig. 1).

To possibly disclose an effect of OSR1 on NaPiIIa, cRNA encoding NaPiIIa was injected into *Xenopus* oocytes with or without cRNA encoding OSR1 and electrogenic phosphate transport determined by the dual-electrode voltage-clamp technique. Addition of phosphate (1 mM) to the bath induced only a small inward current in non-injected or water-injected *Xenopus* oocytes, indicating that *Xenopus* oocytes do not express endogenous electrogenic phosphate transport (Fig. 2). In *Xenopus* oocytes expressing NaPiIIa, phosphate induced an inward current (I_p) consistent with electrogenic entry of Na^+ together with phosphate (Fig. 2). As illustrated in Fig. 2, I_p was significantly enhanced by additional coexpression of OSR1. In contrast, coexpression of the inactive mutant ^{D164A}OSR1 did not significantly modify I_p in NaPiIIa-expressing *Xenopus* oocytes (Fig. 2).

In order to elucidate the *in vivo* significance of OSR1-sensitive renal tubular phosphate transport, experiments were performed in OSR1 knockin mice (*osr1^{tg/+}*), which were heterozygously carrying a WNK-insensitive ^{T185A}OSR1 mutant. The animals were compared to respective wild type mice (*osr1^{+/+}*). On a standard diet, urinary phosphate excretion was significantly higher in *osr1^{tg/+}* mice than in *osr1^{+/+}* mice (Fig. 3). A low-phosphate diet decreased the urinary phosphate excretion in both, *osr1^{tg/+}* and *osr1^{+/+}* mice (Fig. 3). During the low-phosphate-diet, however, urinary phosphate excretion was still significantly higher in *osr1^{tg/+}* mice than in *osr1^{+/+}* mice (Fig. 3).

In theory, the phosphaturia of *osr1^{tg/+}* mice could have been due to an increased serum phosphate concentration. Conversely, phosphaturia due to decreased renal tubular phosphate transport should result in a decrease of serum phosphate concentration. Thus, serum phosphate concentration was determined. As illustrated in Fig. 4, the serum phosphate concentration was significantly lower in *osr1^{tg/+}* mice than in *osr1^{+/+}* mice. Thus, the phosphaturia of *osr1^{tg/+}* mice was not due to hyperphosphatemia but obviously resulted in hypophosphatemia. Dietary phosphate depletion decreased the serum phosphate concentration in both, *osr1^{tg/+}* mice and in *osr1^{+/+}* mice and further enhanced the difference between the genotypes.

To study whether the partial OSR1 deficiency results in lower apical expression of NaPiIIa, BBMVs from the proximal tubule were retrieved and NaPiIIa protein expression determined. As shown in Fig. 5, BBMVs from *osr1^{tg/+}* mice indeed expressed NaPiIIa to a significantly lesser extent than BBMVs from *osr1^{+/+}* mice.

At least in theory, the decreased renal tubular phosphate transport could have resulted from altered hormone levels. Thus, the serum concentration of parathyroid hormone (PTH), calcitriol (1,25(OH)₂D₃), and fibroblast growth factor 23 (FGF23) were determined. As shown in Fig. 6, the serum concentrations of PTH (Fig. 6A) and of 1,25(OH)₂D₃ (Fig. 6B) were similar in *osr1^{tg/+}* mice and in *osr1^{+/+}* mice. The serum FGF23 concentration was, however, significantly higher in *osr1^{tg/+}* mice than in *osr1^{+/+}* mice (Fig. 6C). Dietary phosphate depletion resulted in a significant increase in the 1,25(OH)₂D₃ concentration of both, *osr1^{tg/+}* mice and *osr1^{+/+}* mice and tended to decrease the serum concentrations of PTH and FGF23, effects, however not reaching statistical significance (Fig. 6).

As FGF23 signaling depends on Klotho, additional experiments were performed to elucidate whether partial OSR deficiency modifies renal Klotho expression. To this end, renal Klotho protein was determined in *osr1^{tg/+}* mice and *osr1^{+/+}* mice (Fig. 7). As a result, no significant difference of Klotho protein abundance could be observed between *osr1^{tg/+}* mice and *osr1^{+/+}* mice.

Discussion

The present observations reveal that oxidative stress-responsive kinase 1 (OSR1) is expressed in proximal renal tubules and stimulates renal tubular phosphate transport. Moreover, WNK resistance of OSR1 led to phosphaturia. Accordingly, renal tubular phosphate reabsorption was decreased and thus renal phosphate excretion increased in OSR1 knockin mice carrying one allele of WNK-insensitive ^{T185A}OSR1 mutant (*osr1^{tg/+}*) as compared to the respective wild type mice (*osr1^{+/+}*).

The electrophysiological recordings with the two-electrode voltage clamp in *Xenopus* oocytes heterologously expressing NaPiIIa with and without OSR1 pointed to a stimulatory effect of OSR1 on NaPiIIa activity. Along those lines, partial deficiency of OSR1 activity in *osr1^{tg/+}* mice led to lower NaPiIIa surface abundance in brush border membrane vesicles isolated from the proximal tubule.

The phosphaturia of *osr1^{tg/+}* mice was not secondary to an increased serum phosphate concentration, which was actually decreased. In theory, the phosphaturia *osr1^{tg/+}* could result from an increased serum level of PTH, a hormone increasing urinary phosphate output by stimulation of internalization and subsequent degradation of NaPiIIa^{30;34}. The PTH serum concentration was, however, not significantly different between *osr1^{tg/+}* and *osr1^{+/+}* mice. This observation, does not rule out minor alterations of the serum PTH level. Renal tubular phosphate transport and serum phosphate concentration could further be modified by calcitriol (1,25(OH)₂D₃). The serum 1,25(OH)₂D₃ concentration was, however, again not significantly different between *osr1^{tg/+}* mice and *osr1^{+/+}* mice. The renal 1 α -hydroxylase and thus the formation of 1,25(OH)₂D₃ are stimulated by PTH⁴¹ and by cellular phosphate depletion⁴². The present observations confirm the increase in serum 1,25(OH)₂D₃ following dietary phosphate depletion and confirm the decrease of urinary phosphate output in phosphate-depleted animals.

Our study further shows that the FGF23 serum concentration was higher in *osr1^{tg/+}* mice than in *osr1^{+/+}* mice. FGF23 is known to be up-regulated by 1,25(OH)₂D₃ and in turn to decrease the formation of 1,25(OH)₂D₃^{43;44}. In view of the serum 1,25(OH)₂D₃ levels, the slightly but significantly enhanced FGF23 serum level in *osr1^{tg/+}* mice cannot be explained by 1,25(OH)₂D₃-dependent stimulation of FGF23 expression.

FGF23 is secreted by osteoblasts and decreases renal tubular phosphate transport,⁴⁵. The increased serum FGF23 concentrations could thus contribute to the phosphaturia of the *osr1^{tg/+}* mice. Since FGF23 formation is stimulated by PTH, increased phosphate intake and hyperphosphatemia⁴⁶⁻⁴⁸, the enhanced FGF23 serum level of *osr1^{tg/+}* mice cannot be explained by renal phosphate loss and hypophosphatemia. In theory, the enhanced FGF23 release in *osr1^{tg/+}* mice could be secondary to the effect of OSR1 on NaCl-co-transporter NCC. The carrier is expressed in osteoblasts, fosters bone differentiation and increases bone mineral density²³⁻²⁵. OSR1 activates and phosphorylates NCC^{1-3;9-14}. Alternatively, OSR1 could be effective through inhibition of renal NCC or by more directly modulating FGF23 release in osteoblasts. Further studies are warranted addressing the mechanisms causing the elevated FGF23 plasma concentration in *osr1^{tg/+}* mice.

The effect of FGF23 on renal cells requires expression of Klotho, which thus similarly influences phosphate homeostasis³⁸. The determination of total renal Klotho protein expression did, however not reveal any significant differences between *osr1^{tg/+}* mice and *osr1^{+/+}* mice.

In conclusion, OSR1 participates in the regulation of FGF23 release and renal tubular phosphate transport. OSR1 stimulates NaPiIIa, and partial loss of OSR1 activity leads to phosphaturia and increased formation of FGF23. The present observations thus disclose a completely novel, powerful element in the regulation of mineral metabolism and a novel functional role of oxidative stress-responsive kinase 1.

Acknowledgements

The authors are indebted to Dario Alessi from the MRC Protein Phosphorylation Unit, College of Life Sciences, University of Dundee, Dundee, who kindly provided mice and the OSR1 antibody. They are further grateful to Akiko Saito from the Kyowa Hakko Kirin Co., Ltd., Japan, who provided the antibody against Klotho. The authors further gratefully acknowledge the meticulous preparation of the manuscript by L. Subasic. This work was supported by the Deutsche Forschungsgemeinschaft (DFG) and the Swiss National Center for Competence in Research NCCR Kidney.CH.

Statement of competing financial interests

The authors declare that they have no competing financial interests to disclose.

References

1. Delpire E, Gagnon KB: SPAK and OSR1: STE20 kinases involved in the regulation of ion homoeostasis and volume control in mammalian cells. *Biochem.J* 409:321-331, 2008
2. Kahle KT, Rinehart J, Lifton RP: Phosphoregulation of the Na-K-2Cl and K-Cl cotransporters by the WNK kinases. *Biochim.Biophys.Acta* 1802:1150-1158, 2010
3. Richardson C, Alessi DR: The regulation of salt transport and blood pressure by the WNK-SPAK/OSR1 signalling pathway. *J Cell Sci.* 121:3293-3304, 2008
4. Salter RD, Watkins SC: Dendritic cell altered states: what role for calcium? *Immunol.Rev.* 231:278-288, 2009
5. Solomon A, Bandhakavi S, Jabbar S, Shah R, Beitel GJ, Morimoto RI: *Caenorhabditis elegans* OSR-1 regulates behavioral and physiological responses to hyperosmotic environments. *Genetics* 167:161-170, 2004
6. Wheeler JM, Thomas JH: Identification of a novel gene family involved in osmotic stress response in *Caenorhabditis elegans*. *Genetics* 174:1327-1336, 2006
7. Vitari AC, Deak M, Morrice NA, Alessi DR: The WNK1 and WNK4 protein kinases that are mutated in Gordon's hypertension syndrome phosphorylate and activate SPAK and OSR1 protein kinases. *Biochem.J* 391:17-24, 2005
8. Zagorska A, Pozo-Guisado E, Boudeau J, Vitari AC, Rafiqi FH, Thastrup J, Deak M, Campbell DG, Morrice NA, Prescott AR, Alessi DR: Regulation of activity and localization of the WNK1 protein kinase by hyperosmotic stress. *J Cell Biol.* 176:89-100, 2007
9. Richardson C, Sakamoto K, de los HP, Deak M, Campbell DG, Prescott AR, Alessi DR: Regulation of the NKCC2 ion cotransporter by SPAK-OSR1-dependent and -independent pathways. *J Cell Sci.* 124:789-800, 2011
10. Anselmo LB, Gross JL, Haddad F, Deheinzelin D, Younes RN, Barbuto JA: Functional analysis of cells obtained from bronchoalveolar lavage fluid (BALF) of lung cancer patients. *Life Sci.* 76:2945-2951, 2005
11. Delpire E, Gagnon KB: SPAK and OSR1, key kinases involved in the regulation of chloride transport. *Acta Physiol (Oxf)* 187:103-113, 2006
12. Gimenez I: Molecular mechanisms and regulation of furosemide-sensitive Na-K-Cl cotransporters. *Curr.Opin.Nephrol.Hypertens.* 15:517-523, 2006
13. Huang CL, Yang SS, Lin SH: Mechanism of regulation of renal ion transport by WNK kinases. *Curr.Opin.Nephrol.Hypertens.* 17:519-525, 2008
14. Vitari AC, Thastrup J, Rafiqi FH, Deak M, Morrice NA, Karlsson HK, Alessi DR: Functional interactions of the SPAK/OSR1 kinases with their upstream activator WNK1 and downstream substrate NKCC1. *Biochem.J* 397:223-231, 2006
15. Flatman PW: Cotransporters, WNKs and hypertension: an update. *Curr.Opin.Nephrol.Hypertens.* 17:186-192, 2008
16. Furgeson SB, Linas S: Mechanisms of type I and type II pseudohypoaldosteronism. *J Am.Soc.Nephrol.* 21:1842-1845, 2010

17. Uchida S: Pathophysiological roles of WNK kinases in the kidney. *Pflugers Arch.* 460:695-702, 2010
18. Wilson FH, Disse-Nicodeme S, Choate KA, Ishikawa K, Nelson-Williams C, Desitter I, Gunel M, Milford DV, Lipkin GW, Achard JM, Feely MP, Dussol B, Berland Y, Unwin RJ, Mayan H, Simon DB, Farfel Z, Jeunemaitre X, Lifton RP: Human hypertension caused by mutations in WNK kinases. *Science* 293:1107-1112, 2001
19. Lin SH, Yu IS, Jiang ST, Lin SW, Chu P, Chen A, Sytwu HK, Sohara E, Uchida S, Sasaki S, Yang SS: Impaired phosphorylation of Na⁺-K⁺-2Cl⁻ cotransporter by oxidative stress-responsive kinase-1 deficiency manifests hypotension and Bartter-like syndrome. *Proc.Natl.Acad.Sci.U.S.A* 108:17538-17543, 2011
20. Glover M, O'Shaughnessy KM: SPAK and WNK kinases: a new target for blood pressure treatment? *Curr.Opin.Nephrol.Hypertens.* 20:16-22, 2011
21. Villa F, Goebel J, Rafiqi FH, Deak M, Thastrup J, Alessi DR, van Aalten DM: Structural insights into the recognition of substrates and activators by the OSR1 kinase. *EMBO Rep.* 8:839-845, 2007
22. Villa F, Deak M, Alessi DR, van Aalten DM: Structure of the OSR1 kinase, a hypertension drug target. *Proteins* 73:1082-1087, 2008
23. Biber J, Hernando N, Forster I, Murer H: Regulation of phosphate transport in proximal tubules. *Pflugers Arch.* 458:39-52, 2009
24. Murer H, Forster I, Biber J: The sodium phosphate cotransporter family SLC34. *Pflugers Arch.* 447:763-767, 2004
25. Villa-Bellosta R, Ravera S, Sorribas V, Stange G, Levi M, Murer H, Biber J, Forster IC: The Na⁺-Pi cotransporter PiT-2 (SLC20A2) is expressed in the apical membrane of rat renal proximal tubules and regulated by dietary Pi. *Am J Physiol Renal Physiol* 296:F691-F699, 2009
26. Allon M: Effects of insulin and glucose on renal phosphate reabsorption: interactions with dietary phosphate. *J Am Soc.Nephrol.* 2:1593-1600, 1992
27. DeFronzo RA, Goldberg M, Agus ZS: The effects of glucose and insulin on renal electrolyte transport. *J Clin.Invest* 58:83-90, 1976
28. Feld S, Hirschberg R: Insulinlike growth factor I and the kidney. *Trends Endocrinol.Metab* 7:85-93, 1996
29. Jehle AW, Forgo J, Biber J, Lederer E, Krapf R, Murer H: IGF-I and vanadate stimulate Na/Pi-cotransport in OK cells by increasing type II Na/Pi-cotransporter protein stability. *Pflugers Arch.* 437:149-154, 1998
30. Murer H, Hernando N, Forster I, Biber J: Proximal tubular phosphate reabsorption: molecular mechanisms. *Physiol Rev.* 80:1373-1409, 2000
31. Nowik M, Picard N, Stange G, Capuano P, Tenenhouse HS, Biber J, Murer H, Wagner CA: Renal phosphaturia during metabolic acidosis revisited: molecular mechanisms for decreased renal phosphate reabsorption. *Pflugers Arch.* 457:539-549, 2008

32. Picard N, Capuano P, Stange G, Mihailova M, Kaissling B, Murer H, Biber J, Wagner CA: Acute parathyroid hormone differentially regulates renal brush border membrane phosphate cotransporters. *Pflugers Arch.* 460:677-687, 2010
33. Bacic D, Schulz N, Biber J, Kaissling B, Murer H, Wagner CA: Involvement of the MAPK-kinase pathway in the PTH-mediated regulation of the proximal tubule type IIa Na⁺/Pi cotransporter in mouse kidney. *Pflugers Arch.* 446:52-60, 2003
34. Bacic D, Lehir M, Biber J, Kaissling B, Murer H, Wagner CA: The renal Na⁺/phosphate cotransporter NaPi-IIa is internalized via the receptor-mediated endocytic route in response to parathyroid hormone. *Kidney Int.* 69:495-503, 2006
35. Bhandaru M, Kempe DS, Rotte A, Capuano P, Pathare G, Sopjani M, Alesutan I, Tyan L, Huang DY, Siraskar B, Judenhofer MS, Stange G, Pichler BJ, Biber J, Quintanilla-Martinez L, Wagner CA, Pearce D, Foller M, Lang F: Decreased bone density and increased phosphaturia in gene-targeted mice lacking functional serum- and glucocorticoid-inducible kinase 3. *Kidney Int.* 80:61-67, 2011
36. Dermaku-Sopjani M, Sopjani M, Saxena A, Shojaiefard M, Bogatikov E, Alesutan I, Eichenmuller M, Lang F: Downregulation of NaPi-IIa and NaPi-IIb Na-coupled phosphate transporters by coexpression of Klotho. *Cell Physiol Biochem.* 28:251-258, 2011
37. Foller M, Kempe DS, Boini KM, Pathare G, Siraskar B, Capuano P, Alesutan I, Sopjani M, Stange G, Mohebbi N, Bhandaru M, Ackermann TF, Judenhofer MS, Pichler BJ, Biber J, Wagner CA, Lang F: PKB/SGK-resistant GSK3 enhances phosphaturia and calciuria. *J Am Soc.Nephrol.* 22:873-880, 2011
38. Hu MC, Shi M, Zhang J, Pastor J, Nakatani T, Lanske B, Razzaque MS, Rosenblatt KP, Baum MG, Kuro-o M, Moe OW: Klotho: a novel phosphaturic substance acting as an autocrine enzyme in the renal proximal tubule. *FASEB J* 24:3438-3450, 2010
39. Kempe DS, Dermaku-Sopjani M, Frohlich H, Sopjani M, Umbach A, Puchchakayala G, Capasso A, Weiss F, Stubbs M, Foller M, Lang F: Rapamycin-induced phosphaturia. *Nephrol.Dial.Transplant.* 25:2938-2944, 2010
40. Kempe DS, Ackermann TF, Boini KM, Klaus F, Umbach AT, Dermaku-Sopjani M, Judenhofer MS, Pichler BJ, Capuano P, Stange G, Wagner CA, Birnbaum MJ, Pearce D, Foller M, Lang F: Akt2/PKBbeta-sensitive regulation of renal phosphate transport. *Acta Physiol (Oxf)* 200:75-85, 2010
41. Portale AA, Miller WL: Human 25-hydroxyvitamin D-1alpha-hydroxylase: cloning, mutations, and gene expression. *Pediatr.Nephrol.* 14:620-625, 2000
42. Perwad F, Azam N, Zhang MY, Yamashita T, Tenenhouse HS, Portale AA: Dietary and serum phosphorus regulate fibroblast growth factor 23 expression and 1,25-dihydroxyvitamin D metabolism in mice. *Endocrinology* 146:5358-5364, 2005
43. Razzaque MS, Lanske B: The emerging role of the fibroblast growth factor-23-klotho axis in renal regulation of phosphate homeostasis. *J Endocrinol.* 194:1-10, 2007
44. Tang WJ, Wang LF, Xu XY, Zhou Y, Jin WF, Wang HF, Gao J: Autocrine/paracrine action of vitamin D on FGF23 expression in cultured rat osteoblasts. *Calcif.Tissue Int.* 86:404-410, 2010

45. Amatschek S, Haller M, Oberbauer R: Renal phosphate handling in human--what can we learn from hereditary hypophosphataemias? *Eur.J Clin.Invest* 40:552-560, 2010
46. Lavi-Moshayoff V, Wasserman G, Meir T, Silver J, Naveh-Many T: PTH increases FGF23 gene expression and mediates the high-FGF23 levels of experimental kidney failure: a bone parathyroid feedback loop. *Am J Physiol Renal Physiol* 299:F882-F889, 2010
47. Vervloet MG, van Ittersum FJ, Buttler RM, Heijboer AC, Blankenstein MA, ter Wee PM: Effects of dietary phosphate and calcium intake on fibroblast growth factor-23. *Clin.J Am Soc.Nephrol.* 6:383-389, 2011
48. Wolf M: Forging forward with 10 burning questions on FGF23 in kidney disease. *J Am Soc.Nephrol.* 21:1427-1435, 2010
49. Busch AE, Wagner CA, Schuster A, Waldegger S, Biber J, Murer H, Lang F: Properties of electrogenic Pi transport by a human renal brush border Na⁺/Pi transporter. *J Am Soc.Nephrol.* 6:1547-1551, 1995
50. Rexhepaj R, Dermaku-Sopjani M, Gehring EM, Sopjani M, Kempe DS, Foller M, Lang F: Stimulation of electrogenic glucose transport by glycogen synthase kinase 3. *Cell Physiol Biochem.* 26:641-646, 2010
51. Bohmer C, Sopjani M, Klaus F, Lindner R, Laufer J, Jeyaraj S, Lang F, Palmada M: The serum and glucocorticoid inducible kinases SGK1-3 stimulate the neutral amino acid transporter SLC6A19. *Cell Physiol Biochem.* 25:723-732, 2010
52. Eckey K, Strutz-Seeböhm N, Katz G, Fuhrmann G, Henrion U, Pott L, Linke WA, Arad M, Lang F, Seeböhm G: Modulation of human ether a gogo related channels by CASQ2 contributes to etiology of catecholaminergic polymorphic ventricular tachycardia (CPVT). *Cell Physiol Biochem.* 26:503-512, 2010
53. Rafiqi FH, Zuber AM, Glover M, Richardson C, Fleming S, Jovanovic S, Jovanovic A, O'Shaughnessy KM, Alessi DR: Role of the WNK-activated SPAK kinase in regulating blood pressure. *EMBO Mol.Med.* 2:63-75, 2010
54. Vallon V: In vivo studies of the genetically modified mouse kidney. *Nephron Physiol* 94:1-5, 2003
55. Biber J, Stieger B, Haase W, Murer H: A high yield preparation for rat kidney brush border membranes. Different behaviour of lysosomal markers. *Biochim.Biophys.Acta* 647:169-176, 1981
56. Biber J, Stieger B, Stange G, Murer H: Isolation of renal proximal tubular brush-border membranes. *Nat.Protoc.* 2:1356-1359, 2007
57. Custer M, Lotscher M, Biber J, Murer H, Kaissling B: Expression of Na-P(i) cotransport in rat kidney: localization by RT-PCR and immunohistochemistry. *Am J Physiol* 266:F767-F774, 1994
58. Bachmann S, Schlichting U, Geist B, Mutig K, Petsch T, Bacic D, Wagner CA, Kaissling B, Biber J, Murer H, Willnow TE: Kidney-specific inactivation of the megalin gene impairs trafficking of renal inorganic sodium phosphate cotransporter (NaPi-IIa). *J Am Soc.Nephrol.* 15:892-900, 2004

59. Nedvetsky PI, Tabor V, Tamma G, Beulshausen S, Skroblin P, Kirschner A, Mutig K, Boltzen M, Petrucci O, Vossenkamper A, Wiesner B, Bachmann S, Rosenthal W, Klussmann E: Reciprocal regulation of aquaporin-2 abundance and degradation by protein kinase A and p38-MAP kinase. *J Am Soc.Nephrol.* 21:1645-1656, 2010

Figure legends

Fig. 1. Distribution of the oxidative stress responsive kinase 1 (OSR1) in wild type (*osr1*^{+/+}) kidneys.

A. Confocal images showing double-labeling of OSR1 (green signal) and NaPiIIa (red signals) in proximal tubules from wild type kidneys. Merged images demonstrate co-localization of OSR1 and NaPiIIa in a subset of proximal tubules, whereas some proximal tubules express either OSR1 or NaPiIIa.

B. Higher resolution images demonstrating co-localization of OSR1 (green signal in the apical cellular compartment) and NaPiIIa (red signal in the brushborder membrane) in proximal tubules from wild type kidney; nuclei are counterstained in blue on the merged image.

Fig. 2: Coexpression of OSR1 up-regulates electrogenic phosphate transport in NaPiIIa-expressing *Xenopus* oocytes

A. Representative original tracings showing phosphate (1 mM)-induced current (I_p) in *Xenopus* oocytes injected with water (a) as well as expressing NaPiIIa without (b) or with additional coexpression of wild type OSR1(c) or inactive mutant ^{D164A}OSR1 (d).

B. Arithmetic means \pm SEM (n = 17-62) of phosphate (1 mM)-induced current (I_p) in *Xenopus* oocytes injected with water (H₂O), or expressing NaPiIIa without (2nd bar) or with additional coexpression of wild type OSR1 (3rd bar) or of the inactive mutant ^{D164A}OSR1 (4th bar). *** (p<0.001) indicates statistically significant difference from the absence of OSR1. ### (p<0.001) indicates statistically significant difference between wild type OSR1 and mutant ^{D164A}OSR1.

Fig. 3: Urinary phosphate excretion in *osr1*^{+/+} and *osr1*^{tg/+} mice

Arithmetic means \pm SEM (n = 14-16) of urinary phosphate excretion in heterozygous OSR1 knockin mice (*osr1*^{tg/+}, black bars) and wild type mice (*osr1*^{+/+}, white bars) under normal diet (left bars) and following dietary phosphate depletion (right bars). * (p<0.05), ** (p<0.01) indicate significant difference from *osr1*^{+/+} mice, ### (p<0.001) indicates significant difference from normal diet.

Fig. 4: Serum phosphate concentration in *osr1*^{+/+} and *osr1*^{tg/+} mice

Arithmetic means \pm SEM (n = 14-16) of the serum phosphate concentration in heterozygous OSR1 knockin mice (*osr1*^{tg/+}, black bars) and wild type mice (*osr1*^{+/+}, white bars) under normal diet (left bars) and following dietary phosphate depletion (right bars). * (p<0.05), ** (p<0.01) indicate significant difference from *osr1*^{+/+} mice, # (p<0.05), ## (p<0.01) indicate significant difference from normal diet.

Fig. 5: Protein abundance of renal sodium-dependent phosphate cotransporter NaPiIIa in the brush border membrane in kidneys from *osr1*^{+/+} and *osr1*^{tg/+} mice

Western blot for NaPiIIa. The membrane was stripped and reprobed for β -actin to control for loading. Densitometry was performed and the ratio of the protein of interest over β -actin calculated. Bar graphs present data as arithmetic means \pm SEM (n = 10). *** indicates significant difference from *osr1*^{+/+} mice (Student's t-test, p<0.001).

Fig. 6: Serum concentration of PTH, 1,25(OH)₂D₃, and FGF23 in *osr1*^{+/+} and *osr1*^{tg/+} mice

Arithmetic means \pm SEM (n = 8-11) of serum PTH (A), 1,25(OH)₂D₃ (B), and FGF23 (C) in *osr1*^{+/+} mice (white bars) and *osr1*^{tg/+} mice (black bars) under normal diet (left bars) and following dietary phosphate depletion (right bars). * (p<0.05) indicates significant difference from *osr1*^{+/+} mice, ### (p<0.001) indicates significant difference from normal diet.

Fig.7 Abundance of Klotho protein in the kidneys from *osr1*^{+/+} and *osr1*^{tg/+} mice

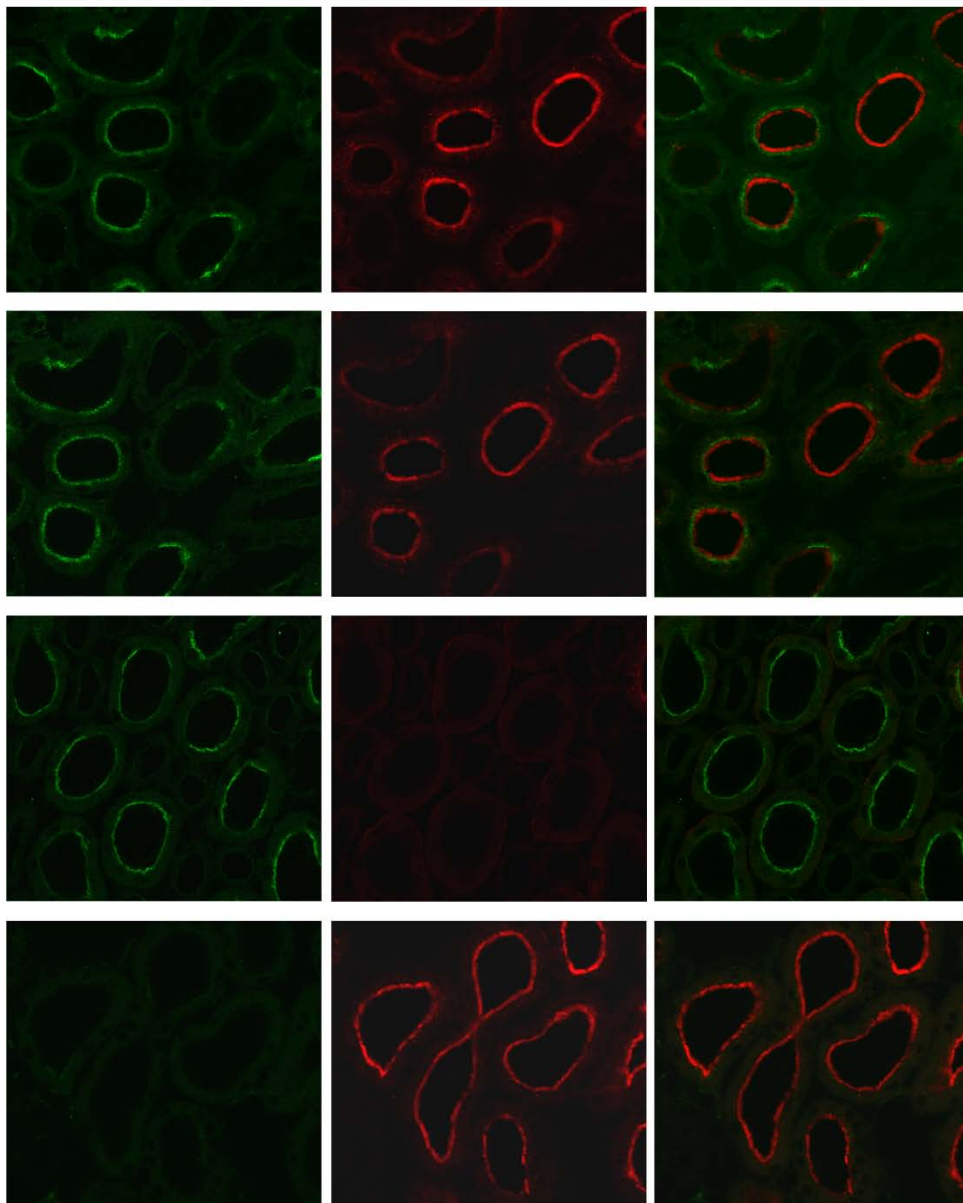
Original Western blots (upper panel) and arithmetic means \pm SEM (n = 10) of the Klotho over GAPDH density ratio in *osr1*^{+/+} mice (white bar) and *osr1*^{tg/+} mice (black bar).

A

OSR1

NaPi-IIa

merge



B

OSR1

NaPi-IIa

merge

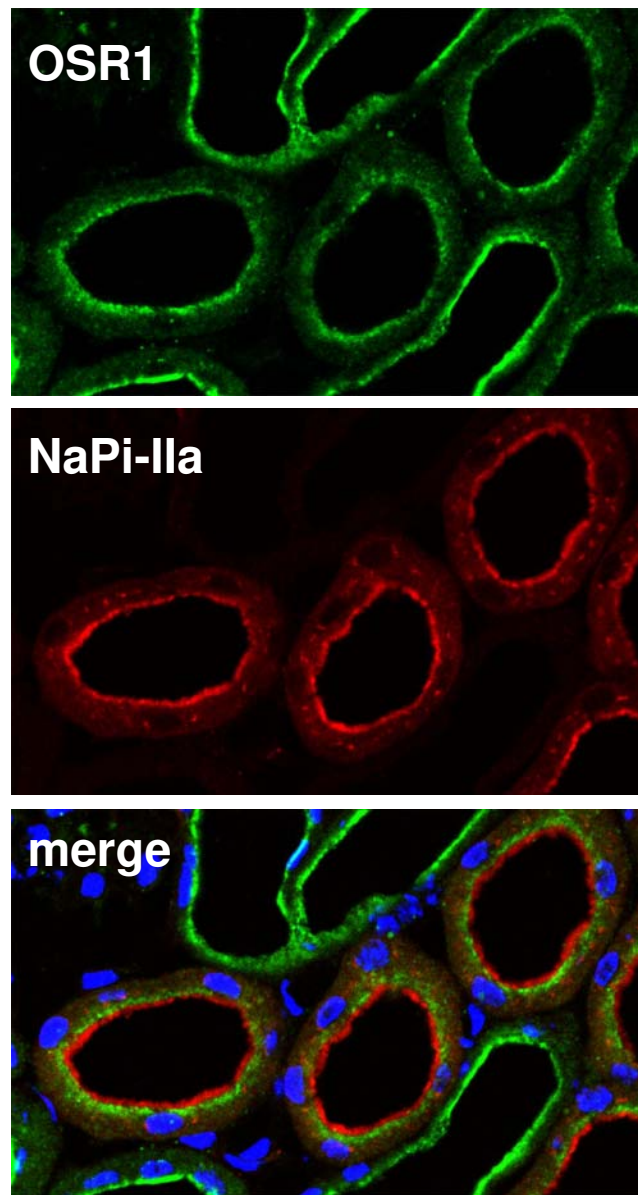


Figure 1

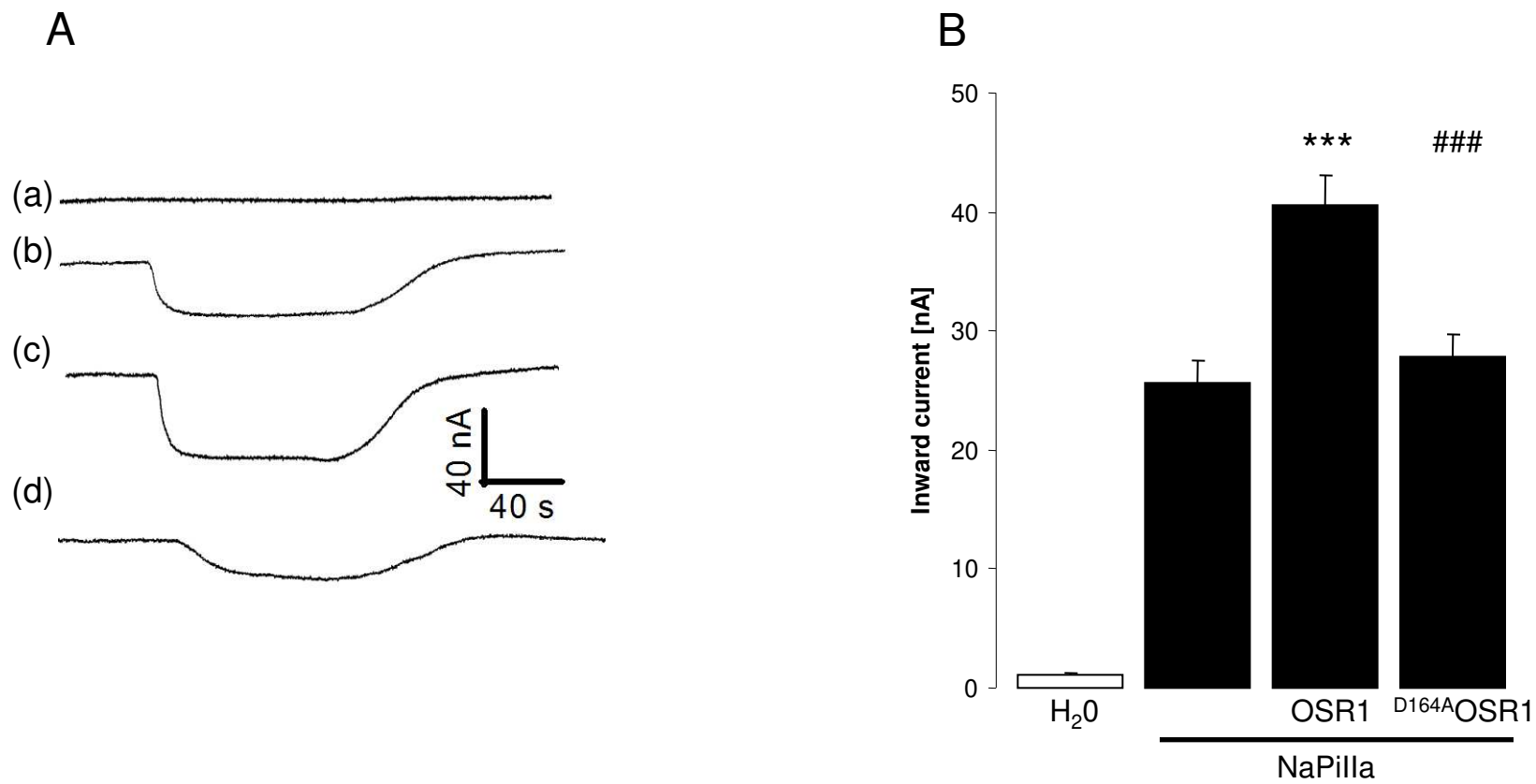


Figure 2

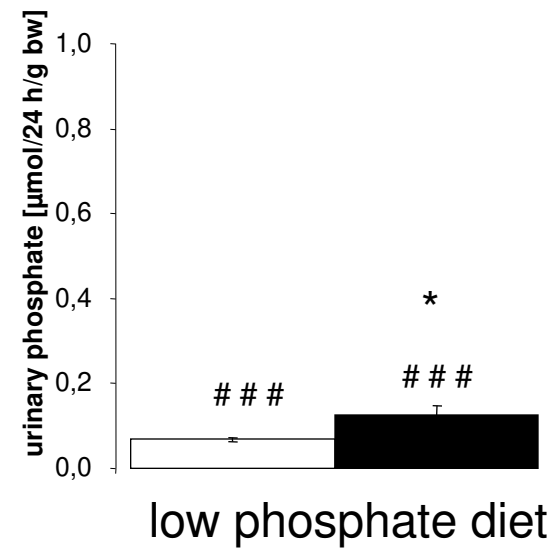
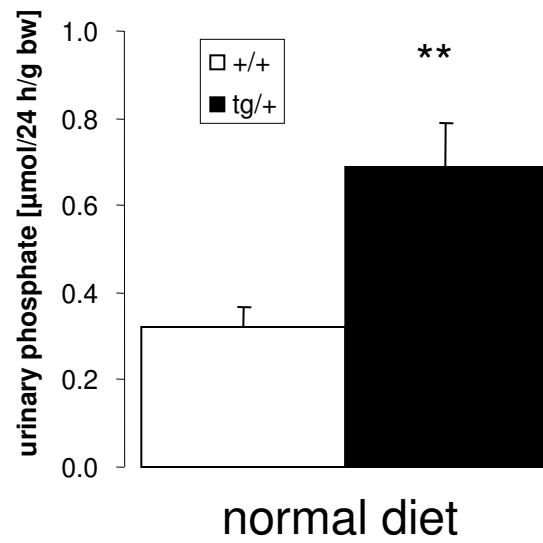


Figure 3

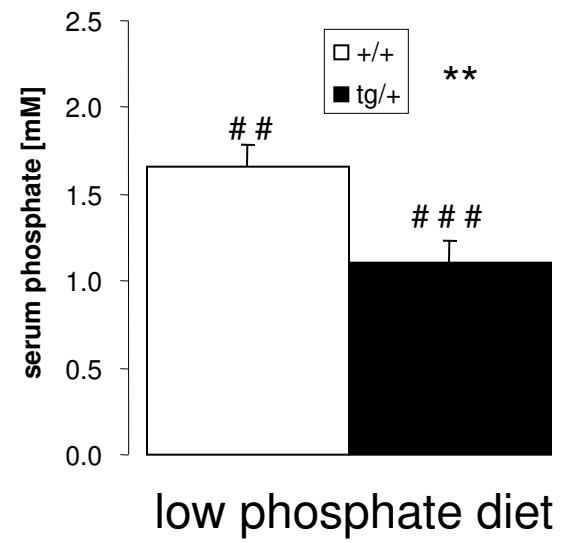
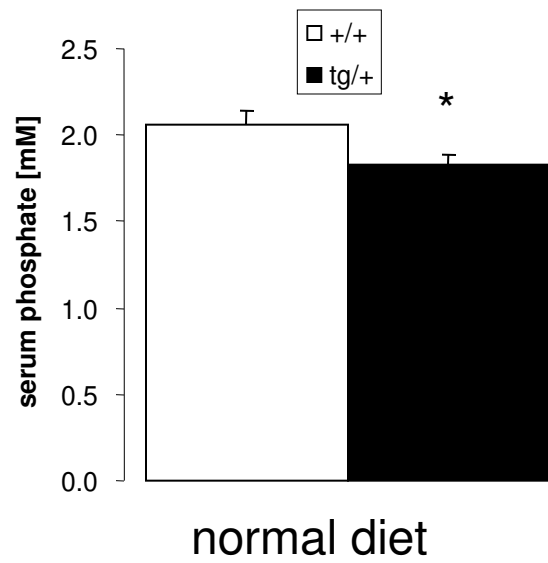


Figure 4

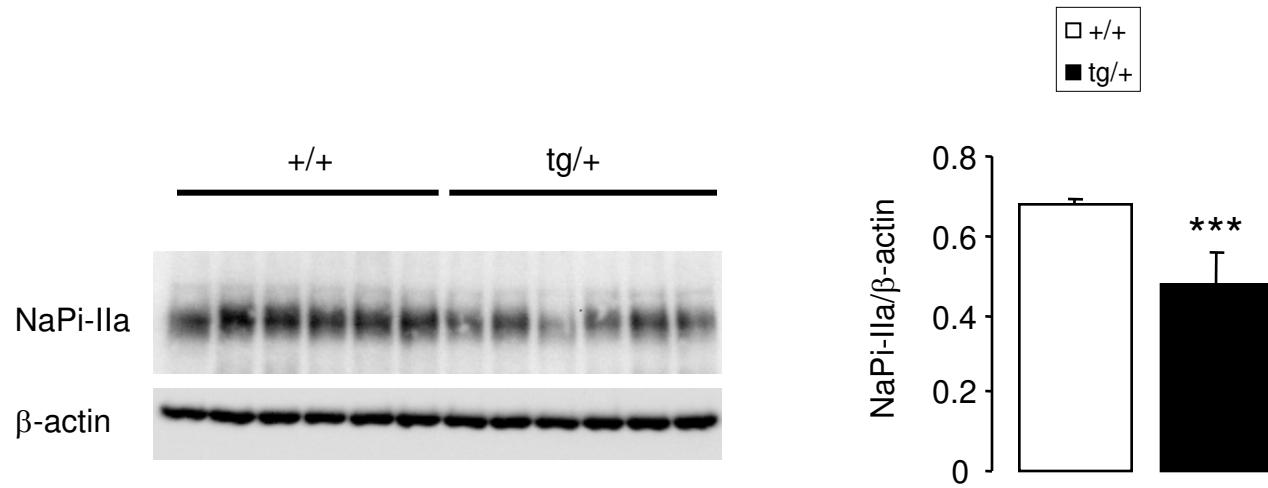
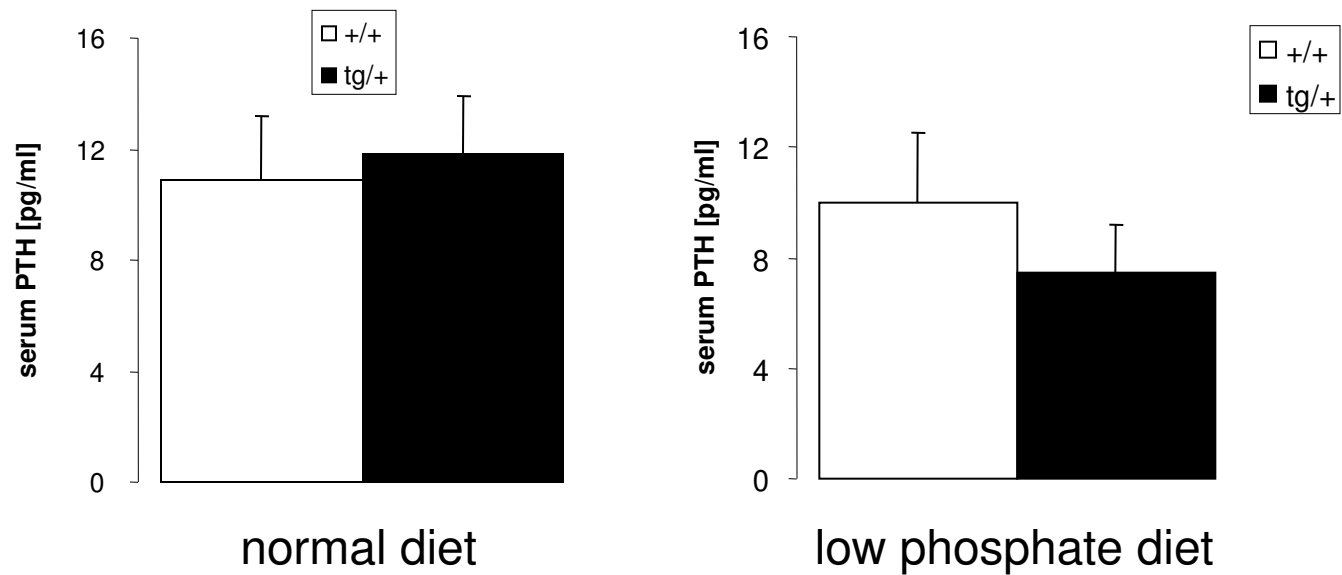


Figure 5

A



B

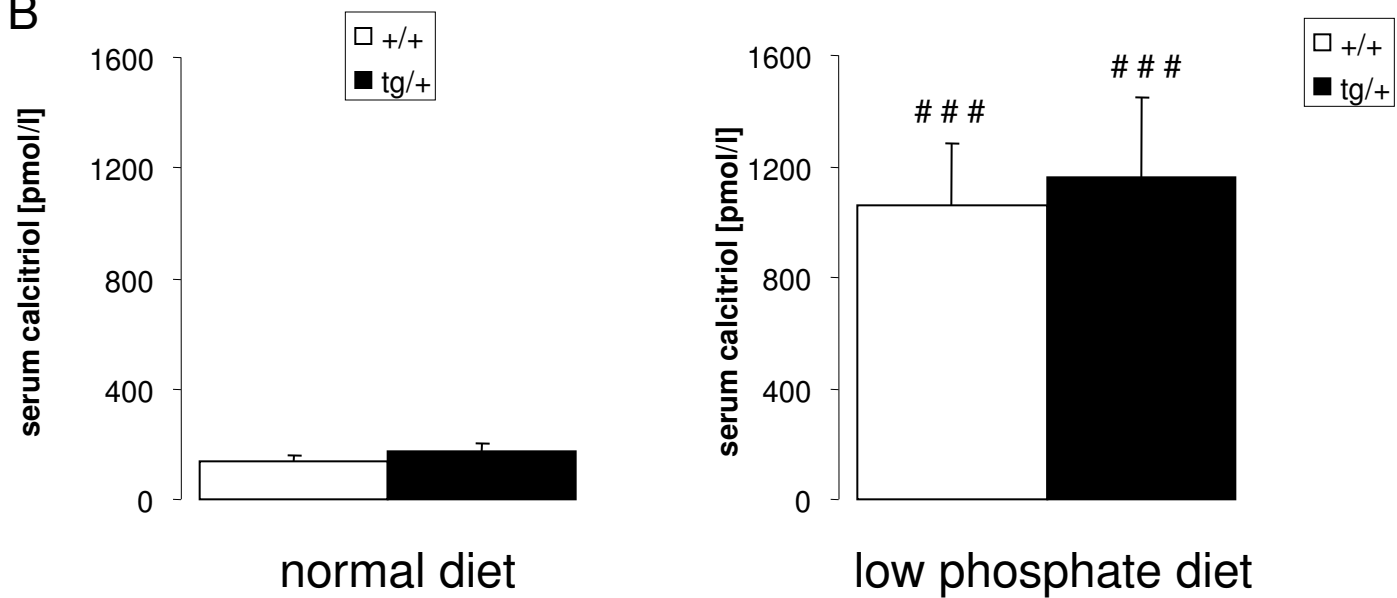


Figure 6

C

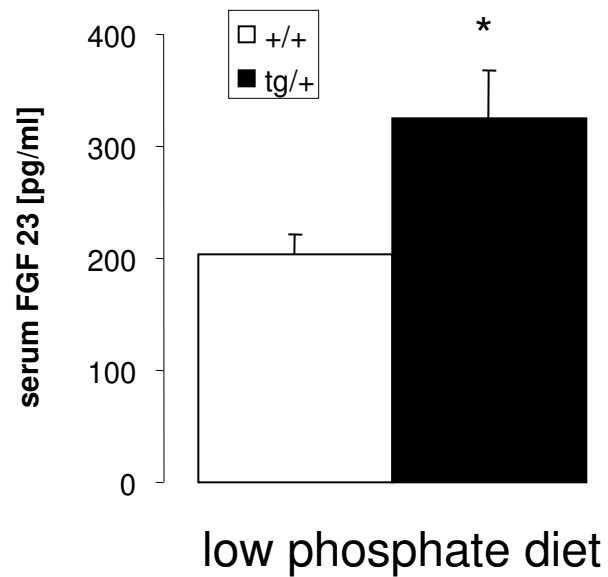
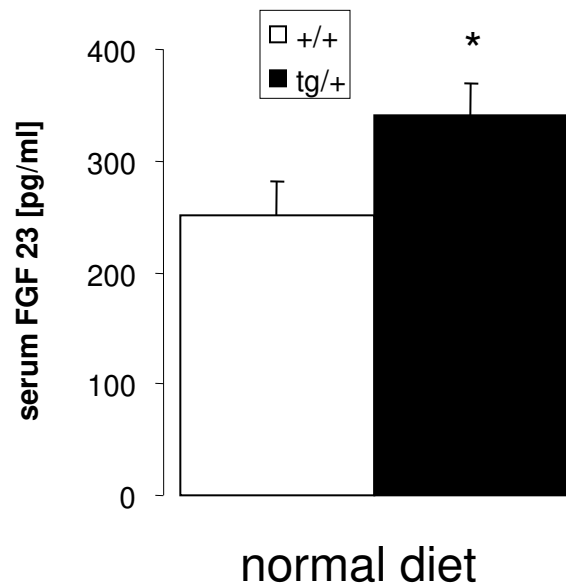


Figure 6

Klotho Protein Expression in OSR1 mice

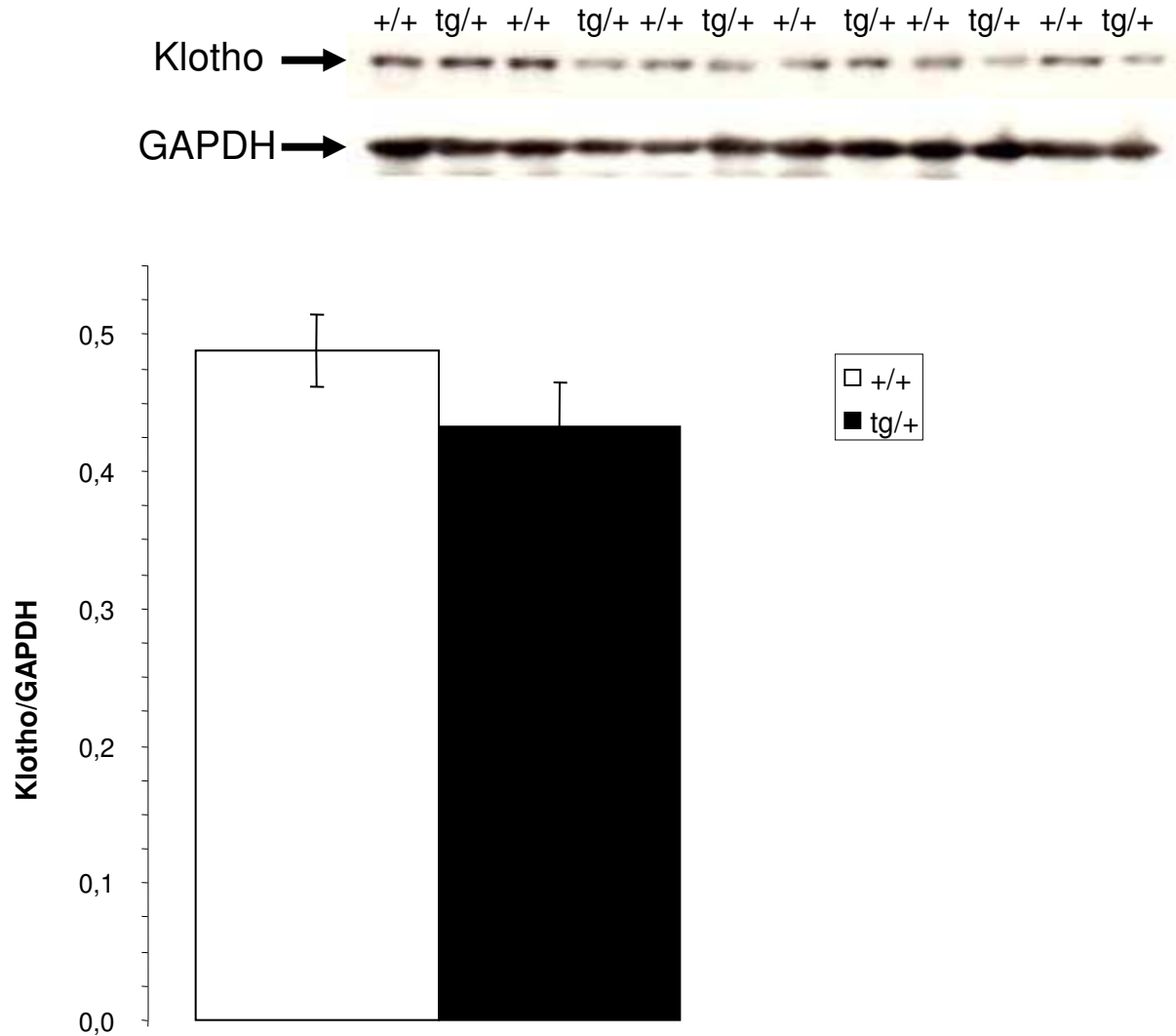


Figure 7